Monitoring of Inosine Monophosphate Dehydrogenase Activity and Expression during the Early Period of Mycophenolate Mofetil Therapy in De Novo Renal Transplant Patients

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Summary: Measurement of inosine-monophosphate dehydrogenase (IMPDH) activity or gene expression was used as a further approach in pharmacokinetics (PK)/pharmacodynamic (PD)-guided mycophenolate mofetil (MMF) therapy. Forty-four de novo kidney transplant patients were enrolled; 35 of these completed the study, and were followed for 24 weeks for clinical status, PK parameters, IMPDH activity and IMPDH1/2 gene expression. IMPDH activity and expression were measured in peripheral blood mononuclear cells before transplant and at week 2, 4, 12 and 24, drawn before (t0) and 2 h (t2h) after MMF administration. No significant correlation was found between IMPDH activity/expression and PK parameters. For both genes, significant enhancement in t2h expression was observed, then decreases towards week 24 with a trend following steroid dosages. Seven patients experienced acute rejection (AR) and exhibited significantly higher pre-transplant expression of both IMPDH1 (median 3.42 vs. 0.84; p = 0.0025), and IMPDH2 genes (135 vs. 104; p = 0.0218) with respect to non-rejecting patients. A significant association was also found between pre-transplant IMPDH1 mRNA and haematological complications (p = 0.032). This study suggests that high steroid dosages may influence IMPDH1/2 expression, hampering their use as a PD biomarker, particularly during the early post-transplant period. The measurement of pre-transplant levels of IMPDH1/2 may contribute to prediction of individual drug responsiveness to improve the clinical management of patients in MMF therapy.

Keywords: IMPDH activity; IMPDH1/2 gene expression; pharmacodynamics; pharmacokinetics; renal transplantation

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Introduction

Mycophenolate mofetil (MMF), the 2,4-morpholino-ester of mycophenolic acid (MPA), is the most frequently prescribed immunosuppressive drug in de novo kidney transplant patients. MMF was promoted as a drug for which no therapeutic drug monitoring (TDM) was considered necessary, although several studies have demonstrated a concentration-effect relationship. Therefore the added value of TDM for MMF is still under debate.

MPA exerts antiproliferative effects on lymphocytes inhibiting inosine 5'-monophosphate dehydrogenase (IMPDH) enzymes; therefore MMF pharmacodynamic (PD) monitoring, such as IMPDH activity measurement, could be an additional approach to tailor and improve MMF therapy. Previous studies have reported that high pre-transplant IMPDH activity in peripheral blood mononuclear cells (PBMCs) is associated with acute rejection (AR) episodes; moreover prolonged exposure to MMF was associated with induction of IMPDH activity, and in long-term patients the activity trend over time seemed to be correlated with AR. This may be partially explained by the up-regulation of the IMPDH encoding genes. Regardless, reports of changes in IMPDH activity after immunosuppressive treatment are conflicting, which has led to the investigations on the impact of immunosuppressive therapy on IMPDH gene expression.

In humans two IMPDH isoforms are encoded by two differently regulated genes (IMPDH1 and IMPDH2). Cell proliferation seems to be related to the up-regulation of IMPDH2, while lymphocyte activation has been associated with an increased expression of both isoenzymes.

Recent studies reported a transient up-regulation of IMPDH1 and a down regulation of IMPDH2 genes, during the first week after transplantation, mainly related to dosage of glucocorticoids. Another report showed an increase in PBMC IMPDH mRNA levels during the first three months after transplantation, which was particularly overexpressed during AR. Moreover, pre-transplant IMPDH1/2 mRNA levels were higher in patients who experienced AR, suggesting that the measurement of basal IMPDH1/2 gene expression may be predictive of therapy efficacy.

MMF administration is frequently associated with adverse events (AE), mainly gastrointestinal or haematological disorders, even though the MPA exposure-side effects relationship still needs to be clearly elucidated, and contradictory results have been reported.

Finally, several studies have demonstrated a large inter-patient variability in basal IMPDH activity, as well as in enzyme inhibition levels and time required for complete recovery of activity after MMF administration. These findings emphasize the role of PD monitoring as a tool to optimize MMF drug dosage and schedule of administration.

The aim of this study was to provide a description of IMPDH activity and expression in the early phase of renal transplantation. A second objective was to identify possible associations between these pharmacodynamic parameters and some clinical factors in order to evaluate the potential use of IMPDH activity and expression as markers to guide MMF therapy.

Methods

Patients and study protocol: We present an observational longitudinal cohort multicenter study (7 Italian centers) in de novo renal transplant recipients from deceased donors. This study was designed in accordance with ICH-GCP (International Conference on Harmonisation-Good Clinical Practice) and the Declaration of Helsinki. The protocol was approved by the local Ethics Committee of each centre, and all patients gave their written informed consent.

MMF treatment (CellCept™, Roche; Basel, Switzerland) treatment was started at 1,000 mg twice daily, and the first oral administration was given within 4 days after transplantation (mean 1 day after, 95% CI 0.89–1.57). The dose could be adjusted by the attending physician based on clinical indicators of drug tolerability and adverse effects.

MMF treatment was part of an immunosuppressive regimen consisting of antibody preparations (Basiliximab and/or Thymoglobulin) as induction therapy, cyclosporine (CsA) and steroids, according to the manufacturer’s instructions.

In particular, thymoglobulins were infused at 0.3–1.0 mg/kg/day dose for 2 to 4 days from transplant and Basiliximab was administered at 20 mg dose, at the beginning of surgery and on postoperative day 4. From the transplant day on, methylprednisolone was started at 5 mg/kg/day i.v. tapered within the first months to 0.2 mg/kg/day p.o. and maintained at 0.05 mg/kg/day thereafter.

Type, dosing and administration of induction therapy as well as the choice of sampling times for CsA levels [concentration just before (C0) and/or 2 h (C2h) after MMF administration] were scheduled as per local practice. Quantification of CsA blood concentration, sample handling and storage procedure were performed in each centre according to the instructions of the commercially obtained antibody-conjugated magnetic immunoassay (ACMIA, Siemens Healthcare Diagnostics, Deerfield, IL, USA). An integral element for further quality assurance was the continuous participation in the international proficiency testing scheme for CsA measurements.

Among the main co-medications used there were sulfamethoxazole and trimethoprim for standard prophylactic treatment of Pneumocystis carinii, ganciclovir for cytomegalovirus therapy, calcitriol for osteoporosis, and low molecular weight heparins for thrombosis prophylaxis. Some patients also received antibiotics (ciprofloxacin, cephalixin, cefazidime), antihypertensive drugs (calcium channel blockers, angiotensin-converting enzyme inhibitors) and furosemide as diuretic therapy.

Rejection diagnosis was considered in patients with an increase in serum creatinine of more than 25% from the baseline level, and oliguria. All ARs were confirmed by a core biopsy performed within 48 h and graded according to the Banff criteria.

Rejection episodes were first-line treated with three to five pulse steroid intravenous injections of 500 mg/day tapered to 125 mg/day. In the case of steroid resistance, AR patients were further treated with antithymocyte/antilymphocyte globulin preparations, or with monoclonal antibodies targeted to the CD3 receptor (Muromonab-CD3), as per local practice. Anyway, no MMF dose increase was considered in the event of an AR requiring steroid treatment.

Adverse events (AE) were graded according to the standard response and toxicity criteria. Patients were monitored for 24 weeks by collecting clinical information which was systematically provided in a data collection sheet; this included data on clinical status, concomitant medications and changes in dosages of each drug administered, occurrence of adverse events, infections, and graft rejection. All patients were also monitored for plasma total/free MPA concentrations, IMPDH activity, IMPDH1/2 genes...
expression and other hematocellular parameters, before transplantation (week 0) and at 2, 4, 12 and 24 weeks thereafter.

Plasma and PBMC samples for determination of MPA total and free concentrations, as well as IMPDH activity and expression were collected at each centre according to a shared standardized procedure, frozen at –80°C, then shipped on dry ice to the Foundation Policlinico S. Matteo of Pavia, for sample analysis.

Blood collection and isolation of lymphocytes: Blood samples were collected in tubes containing ethylenediamine tetraacetic acid (EDTA) and stored at room temperature. Promptly after blood drawing, PBMCs were isolated by the Ficoll gradient density method. PBMCS collected were incubated at 4°C for 10 min with 3 mL of a red blood cells lysis buffer (150 mM NH4Cl, 10 mM KHCO3, 0.1 mM disodium EDTA, pH 7.3) and centrifuged at 900 × g for 15 min at 4°C. For the IMPDH activity assay, pellets were stored at –80°C until assay. Samples under these conditions are stable up to one year, as previously reported. For RNA extraction, PBMCs were immediately placed in 2X Lysis Buffer (Applied Biosystems, Carlsbad, USA) and stored at –80°C.

Pharmacokinetics of MPA and IMPDH activity: For pharmacokinetic (PK) evaluation, a total of three EDTA blood samples were drawn at each follow-up visit just before the morning dose (C0), and 40 min (C0.67h) and 2 h (C2h) after MMF administration. Sampling times were chosen considering the pharmacokinetic characteristics of MMF and the validated algorithm for quantification of MPA area under the concentration-time curves from 0 to 12 h (AUC0–12h). Just after drawing blood, samples were chilled and promptly centrifuged at 4°C to avoid deglucuronidation of metabolites, and plasma total (totMPA) and free MPA (fMPA) concentrations were quantified by a validated HPLC method, as previously reported. For total MPA, in the concentration range from 0.1 to 15 µg/mL the calibration curves were linear and r2 ranged from 0.9961 to 0.9980; the LLOQ was 0.0125 µg/mL; the inter- and intraday CVs were 5%. IMPDH activity of these samples, regularly assayed up to 1 year later, are stable up to one year, as previously reported. For RNA extraction, PBMCs were immediately placed in 2X Lysis Buffer (Applied Biosystems, Carlsbad, USA) and stored at –80°C.

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Pharmacokinetic parameters such as the concentration just before (C0), and 40 min (C0.67h) and 2 h (C2h) after MMF administration were assessed for both totMPA and fMPA. The totMPA drug exposure was expressed as the area under the concentration-time curve from 0 to 12h (AUC0–12h) and was calculated using the following algorithm:

\[
AUC_{0–12h}(\text{mg h/L}) = 7.182 + 4.607C_0 + 0.998C_{0.67h} + 2.149C_{2h}
\]

Apparent oral clearance (CL/F=dose/AUC0–12h) was calculated by normalizing MMF dose to the subject’s body weight and multiplying it by the ratio of MPA molecular weight to MMF molecular weight (0.739). The fraction of MPA unbound in plasma (fu%) was determined as IMPA/total MPA.

For pharmacodynamic evaluation, PBMCs from the same drawn blood samples used for PK were used to measure also IMPDH activity. These sampling times were chosen to facilitate treatment on an outpatient basis, and considering that IMPDH inhibition measured 2 h after MMF administration is about 75–80% of its nadir, usually reported at 1 h. IMPDH activity was determined in PBMCs at time 0 (t0) and 2 h (t2h) after MMF administration, by a HPLC method based on the rate of XMP production, as previously validated and reported. Calibration curve of XMP was linear, with a r2 ranging from 0.9961 to 0.9980; the LLOQ was 0.04 nmol, and the inter- and intraday CVs was <5%. IMPDH activity was repeatedly measured in a pool of PBMCs from healthy subjects stored at –80°C as internal quality controls. The enzyme activity of these samples, regularly assayed up to 1 year later, exhibited a mean CV of 4%. The same reaction samples run up to 6 h after boiling showed a coefficient of variability of <2%.

Real time quantitative polymerase chain reaction (RT-qPCR): IMPDH1 and IMPDH2 gene expression was determined in PBMCs at t0 and t2h, using the relative quantification technique with RT-qPCR. Total RNA was extracted from PBMCs using the ABI PRISM 6100 platform (Applied Biosystems) according to the manufacturer’s instructions. For cDNA synthesis, reverse transcription was performed on 500 ng of RNA in the presence of random hexamers using M-MLV reverse transcriptase (High Capacity cDNA Archive Kit, Applied Biosystems).

For the IMPDH1 and IMPDH2 gene expression profiling, specific FAM-MGB Assay-on-Demand was used with probes placed at the exon/exon boundaries (Hs01597683, Hs00168418m1, respectively). As a reference gene (HKG), VIC-MGB labelled glyceraldehyde-3-phosphate dehydrogenase (NM_002046; PN4326317E) was used. As a control, Universal Human RNA (Ambion, Applied Biosystems) was used. RT-q-PCR was carried out on an ABI PRISM 7900HT (Applied Biosystems). Reactions were performed in a final 20 µL 1× TaqMan Universal Master Mix. After two incubation steps (50°C 10 min, 95°C 2 min), the thermal cycling protocol was 40 PCR cycles (95°C 30 s, 60°C 1 min).

The relative amount of target RNA was determined using ABI PRISM 7900HT SDS 2.2 software and the comparative method (2–ΔΔCt). The data are expressed as the mean fold change of a given gene in comparison with the reference gene. Each patient sample was run in triplicate.

Statistical analyses: The Shapiro-Wilk’s W test was used to evaluate data distribution. Descriptive results are presented as the means and standard deviation (SD) and 95% confidence intervals (CI) or medians and 95% CI if data were not normally distributed. Inferential univariate analysis using either parametric or non-parametric tests was used, as appropriate for the data distribution. Correlations between the PK/PD and clinical parameters associated with MPA administration were evaluated with the Pearson correlation coefficient.

Linear regression models for repeated measures (after dependent variable log-transformation) were used to analyze changes over time at C0, C2h for totMPA and fMPA, AUC0–12h for totMPA, IMPDH1 and IMPDH2 genes expression and IMPDH activity, serum creatinine, creatinine clearance (CrCL), lymphocytes, albumin, hemoglobin, and hematocrit. Data from patients lost at follow-up were included in the study.
statistical analysis until the week before their exit from the study.

A conventional receiver-operating characteristic (ROC) curve was used to analyze IMPDH activity and IMPDH1/2 gene expression to determine the cut-off points yielding the highest combined sensitivity and specificity to predict which patients were likely to suffer AR.26

A p value <0.05 was deemed statistically significant and all tests were two-sided. Data analysis was performed with the STATA statistical package (Stata Corporation, College Station, Texas, USA).

The sample size was chosen with respect to the exploratory nature of this study; it was powered considering a coefficient of correlation r equal to 0.4 as the minimal limit acceptable in order to judge as good two variable correlation. With a sample size of forty, the linear regression test would have 85% power to detect a −0.4 (α = 0.05 one-side) coefficient of correlation, which would be statistically significant. Considering a 10% drop-out, forty-five patients should be enrolled.

Results

Forty-four patients were enrolled, and 35 (77.7%) completed the study. Nine patients were withdrawn prematurely from the study: one died from a non-study related event, four were switched to tacrolimus after evidenced AR, two suffered gastrointestinal adverse events, one suffered graft loss, and one withdrew their informed consent.

The demographic and baseline characteristics of the patient population are shown in Table 1.

The aetiology of renal failure covered a broad range of disorders, but all patients were on dialysis, mainly haemodialysis, for variable periods of time (4 months to 12 years). Most had one or more risk factors for rejection, such as HLA mismatching: at least one mismatch for each type of HLA antigen was ascertained in 52% of patients. Concerning induction therapy, 34 out of 44 patients were treated with basiliximab (77.2%), five with thymoglobulin (11.4%) and the other five with both basiliximab and thymoglobulin (11.4%). Laboratory results for each visit are summarized in Table 2.

Pharmacokinetic monitoring: The mean MMF dose administered during the follow-up period was 1849 ± 357 mg/day (28 ± 7 mg/kg/day). Table 3 summarizes the main PK parameters of MPA, the t0 and t2h IMPDH activity and IMPDH1/2 gene expression values, as well as the median dose of steroids at each scheduled visit. At all follow-up visits, about 71% of C0totMPA levels fell within the proposed therapeutic range for renal transplantation (1–3.5 µg/mL)27 and about 60% for AUC0–12h values (30–60 µg h/mL).28–30

AUC0–12h and C0 values did not show statistically significant differences during the follow-up. However, the intermittent variability was rather marked both for C0 totMPA (CV% 90.9%; range 0.056–8.34 µg/mL) and C0 fMPA (90.1%, range 0.013–0.147 µg/mL). The AUC0–12h, intermittent variability was 45.5% (range 14.0–96.2 µg/h/mL). For C0 totMPA concentrations, the mean intrapatient coefficient of variation was 51% (95% CI 43.8–58.3), and for the C0 fMPA it was 42.8% (95% CI 35.2–50.4), whereas a mean intrapatient coefficient of variation of 28.6% (95% CI 23.8–33.5) was observed for MPA AUC0–12h.

Pharmacodynamic monitoring: IMPDH activity in PBMCs was measured before transplant and at each scheduled visit, before (t0) and 2 h (t2h) after MMF administration. Pre-transplant IMPDH activity ranged from 0.21 to 27.8 nmol/h/mg, with an interpatient CV of 103.0%. This variability remained largely unchanged during the follow-up. The median intrapatient variability both at t0 and t2h was used to analyze IMPDH activity and

Table 1. Demographic and laboratory data on enrolled patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients (n = 44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years; median, range)</td>
<td>47 (27–64)</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>20/24</td>
</tr>
<tr>
<td>Weight (kg; mean ± SD)</td>
<td>68.6 ± 11.5</td>
</tr>
<tr>
<td>BMI (kg/m2; mean ± SD)</td>
<td>24.2 ± 3.7</td>
</tr>
<tr>
<td>Primary renal disease</td>
<td></td>
</tr>
<tr>
<td>Uncertain</td>
<td>12 (27.3%)</td>
</tr>
<tr>
<td>Chronic/membranoproliferative, glomerulonephritis</td>
<td>5 (11.4%)</td>
</tr>
<tr>
<td>IgA nephropathy</td>
<td>4 (9.1%)</td>
</tr>
<tr>
<td>Proliferative/cystic kidney disease</td>
<td>8 (18.2%)</td>
</tr>
<tr>
<td>Nephroangiosclerosis due to hypertension</td>
<td>2 (4.6%)</td>
</tr>
<tr>
<td>Renal vascular disease: unspecified</td>
<td>2 (4.6%)</td>
</tr>
<tr>
<td>Other</td>
<td>11 (24.8%)</td>
</tr>
<tr>
<td>Time on dialysis before transplantation (months; median-range)</td>
<td>32 (4–144)</td>
</tr>
<tr>
<td>Type of dialysis</td>
<td></td>
</tr>
<tr>
<td>% haemodialysis</td>
<td>36 (81.8%)</td>
</tr>
<tr>
<td>% peritoneal dialysis</td>
<td>8 (18.2%)</td>
</tr>
<tr>
<td>HLA mismatching (A+B) (mean ± SD)</td>
<td>1.29 ± 0.62</td>
</tr>
<tr>
<td>HLA mismatching (DR) (mean ± SD)</td>
<td>0.80 ± 0.56</td>
</tr>
<tr>
<td>Induction therapy (n of patients, %)</td>
<td></td>
</tr>
<tr>
<td>Thymoglobulin</td>
<td>5 (11.4%)</td>
</tr>
<tr>
<td>Basiliximab</td>
<td>34 (77.2%)</td>
</tr>
<tr>
<td>Thymoglobulin/Basiliximab</td>
<td>5 (11.4%)</td>
</tr>
<tr>
<td>Serology status (% of patients)</td>
<td></td>
</tr>
<tr>
<td>Cytomegalovirus positivity</td>
<td>35 (79.6%)</td>
</tr>
<tr>
<td>Epstein-Barr virus positivity</td>
<td>25 (56.8%)</td>
</tr>
</tbody>
</table>

Table 2. Laboratory results of the analyzed patients at each visit

<table>
<thead>
<tr>
<th></th>
<th>Week 0 (n = 44)</th>
<th>Week 2 (n = 44)</th>
<th>Week 4 (n = 42)</th>
<th>Week 12 (n = 35)</th>
<th>Week 24 (n = 35)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/L)</td>
<td>38 ± 6</td>
<td>35 ± 5</td>
<td>38 ± 5</td>
<td>40 ± 5</td>
<td>42 ± 5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total bilirubin (mg/L)</td>
<td>5.2 ± 2.5</td>
<td>8.2 ± 5.2</td>
<td>7.6 ± 3.4</td>
<td>7.0 ± 0.34</td>
<td>8.2 ± 4.6</td>
<td>0.012</td>
</tr>
<tr>
<td>Serum creatinine (mg/L)</td>
<td>6.9 ± 2.3</td>
<td>2.3 ± 2.1</td>
<td>1.7 ± 0.8</td>
<td>1.6 ± 0.9</td>
<td>1.4 ± 0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CrCl (/mL/min)</td>
<td>13.2 ± 8.2</td>
<td>51.3 ± 24.8</td>
<td>55.7 ± 31.4</td>
<td>60.8 ± 23.6</td>
<td>64.9 ± 22.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Estimated GFR (/mL/min)</td>
<td>9.3 ± 5.7</td>
<td>44.6 ± 21.7</td>
<td>49.1 ± 18.9</td>
<td>53.2 ± 22.1</td>
<td>55.1 ± 17.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urine protein (mg/L)</td>
<td>n.a.</td>
<td>181 ± 308</td>
<td>115 ± 169</td>
<td>129 ± 238</td>
<td>105 ± 192</td>
<td>0.549</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>121 ± 20</td>
<td>106 ± 16</td>
<td>115 ± 15</td>
<td>125 ± 15</td>
<td>130 ± 16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>36.5 ± 6.6</td>
<td>32.1 ± 5.1</td>
<td>34.6 ± 4.1</td>
<td>37.7 ± 4.4</td>
<td>38.7 ± 4.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lymphocytes (x10⁶/mL)</td>
<td>5.3 ± 8.6</td>
<td>7.5 ± 9.4</td>
<td>8.7 ± 10.7</td>
<td>9.4 ± 10.1</td>
<td>11.4 ± 12.9</td>
<td>0.108</td>
</tr>
</tbody>
</table>

Data are mean ± SD.
*p<0.05
*One-way analysis of variance.
*Creatinine clearance was calculated with the Cockcroft-Gault formula.
*Estimated using the MDRD formula.

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was 61.0% (95% CI 31.0–90.6%). IMPDH inhibition levels, reported as the percentage of residual activity 2 h after MMF administration, exhibited wide variability, with a median of 73.9% (95% CI 56.1–102.9). No significant correlations were found between t₀ IMPDH activity and C₀ totMPA or fMPA, nor between IMPDH inhibition level and C₂₅ totMPA or fMPA.

A significant positive correlation was found between t₀ IMPDH activity and the corresponding week of treatment, with an increasing trend over time from 2.9 mmol/h/mg (95% CI 2.3–3.5) at week 0, to 5.2 mmol/h/mg (95% CI 4.3–10.0) at week 24 (p = 0.014) (Fig. 1 and Table 3).

No significant differences in lymphocyte or protein concentration, used to normalize the IMPDH activity, were observed between patients with or without thymoglobulin induction therapy nor between patients with or without AR (data not shown).

**IMPDH1** gene expression showed a high pre-transplant inter-individual variability (CV 130%) whereas the variability of **IMPDH2** was rather low (17%). Moreover, pre-dose **IMPDH1** showed a variability which greatly increased at week 2 (589%), returning to baseline levels at week 24 (150%). In addition, 2 h after MMF administration both **IMPDH1** and **IMPDH2** gene expression levels were significantly enhanced (up to 5–6 orders of magnitude and 1–2 orders of magnitude for **IMPDH1** and **IMPDH2**, respectively). The t₀ expression of both genes was higher at week 2, but significantly decreased towards week 24 (p = 0.0011 and p = 0.0003 for **IMPDH1** and **IMPDH2**, respectively), with a trend that followed the decrease in steroid dosage (Fig. 2 and Table 3). On the other hand, no correlation was found between **IMPDH1** or **IMPDH2** and totMPA (**IMPDH1**: r = 0.05, p = 0.57; **IMPDH2**: r = 0.28 p = 0.43), IMPA (**IMPDH1**: r = 0.39, p = 0.53; **IMPDH2**: r = 0.25, p = 0.42), or UAC₀.₁₂₅b (**IMPDH1**: r = −0.07 p = 0.58; **IMPDH2**: r = 0.07 p = 0.57). Moreover, neither the t₀ or t₂₀ IMPDH activity showed any correlation with **IMPDH1** (t₀: r = −0.06 p = 0.41; t₂₀: r = −0.02 p = 0.80) or **IMPDH2** (t₀: r = 0.13 p = 0.19; t₂₀: r = −0.14 p = 0.09).

**Clinical outcome:** Seven patients experienced one AR episode each; in five the AR occurred within the first two weeks after transplantation, in the other two, between weeks 2 and 4. MPA levels exhibited no significant differences between AR and non-AR patients (p = 0.48); C₀ totMPA and UAC₀.₁₂₅b values mainly fell within the suggested therapeutic range (C₀: median 1.85, 95% CI 1.24–2.26; UAC₀.₁₂₅b: median 38.64, 95% CI 32.5–44.3). Furthermore no significant difference was found between AR and non-AR patients in CsA₀ or C₂₅ levels (median 259.5, 95% CI 230.1–304.7 for AR patients, vs. median 216.0, 95% CI 139.1–324.1 for non-AR patients, p = 0.28; C₂₅: median 1,186.0, 95% CI 1,011.7–1,426.5 for AR patients, vs. median 942.0, 95% CI 649.5–1,735.2 for non-AR patients, p = 0.94).

No association between potential risk factors of rejection (primary renal disease, type and time on dialysis before transplantation, type of induction therapy, HLA mismatch, and serology status) and the occurrence of rejection was found. No differences in t₀ or t₂₀ IMPDH activity levels were found between AR and non-AR patients.

**IMPDH1** and **IMPDH2** gene expression levels during AR did not significantly differ from levels before AR or from those of patients without AR. On the contrary, patients who underwent AR exhibited significantly higher pre-transplant **IMPDH1** (median

![Fig. 1. Variation of pre-dose (t₀) IMPDH activity over time during the monitoring period](image-url)
3.42, 95% CI 1.49–10.90 for AR patients, vs. median 0.84, 95% CI 0.39–1.34 for non-AR patients; \(p=0.0025\), and barely significantly higher IMPDH2 (median 135, 95% CI 102–173 for AR patients, vs. median 104, 95% CI 102–110 for non-AR patients; \(p=0.0218\) (Figs. 3A and 3B).

The ROC analysis of IMPDH1 levels showed a significant AUC of 0.865 (95% CI 0.72–0.95, \(p=0.0001\)) (Fig. 3C). A cut-off value of 1.36 for IMPDH1 had a specificity of 71.4% (95% CI 53.7–85.3%) and a sensitivity of 100% (95% CI 58.9–100%) with a likelihood ratio of 3.5.

All patients experienced at least one adverse event, gastrointestinal AE (diarrhoea, nausea, vomiting) in 17 cases, hematological AE (leukopenia, anemia) in 19, and cytomegalovirus infection in 11. A significant association was observed between pre-dose PK parameters and gastrointestinal AE (the best with \(C_0\) totMPA, \(p=0.0018\)) (Fig. 4A). On the other hand, except for pre-transplant IMPDH1 and hematological complications (median 0.30, 95% CI 0.07–4.87 for patients with, vs. median 1.32, 95% CI 0.64–2.96 for patients without; \(p=0.032\)) (Fig. 4B), no significant association between AE and IMPDH activity or IMPDH1 and IMPDH2 was found.

**Discussion**

Several studies have reported a large intra- and interpatient variability in MPA pharmacokinetics and IMPDH activity,\(^{3,4,6,11,28}\) reasonably associated with both genetic (e.g., polymorphisms of IMPDH1 gene,\(^{31}\)) and transporters, e.g., multidrug resistance-associated protein 2 (MRP2)\(^{23}\) and UDP-glucuronosyltransferases\(^{33}\)) and environmental factors (e.g., infections). These findings emphasize the potential role of PD monitoring as a tool to optimize MMF drug dosage and to refine the schedule of administration. Thus, new approaches reflecting individual responses are required to monitor specific markers of the biological effects of immunosuppressive drugs.

Although there is uniformity in finding that IMPDH activity and MPA concentrations during the dosing interval are inversely proportional regardless of drug formulation administered (mycophenolate mofetil or enteric-coated mycophenolate sodium), some differences were highlighted in the correlations between the different PK/PD examined parameters.\(^{23,34,35}\) Several studies and strategies were suggested to individualize MPA therapy and improve clinical outcome. Among proposed strategies, the
have been reported for transplant patients has been con
have reported an up-regulation, whereas others have described
algorithm for an early post-transplant period in kidney trans-
study, the sample size used to describe pharmacokinetic parameters
were chosen considering a three-point sampling strategy within
first 2 h after drug intake, to facilitate treatment on an outpatient
basis. For this reason, we used a previously validated sampling
algorithm for an early post-transplant period in kidney trans-
plantation.

In subjects with normal renal function, the MPA free fraction
(fu%) has been reported between 1 and 2.5%, but increasing up
to 7% in patients with impaired renal function. A reduction
in renal function has been associated with decreased total MPA
exposure as a result of displacement of MPA from plasma protein-
binding sites by increasing MPA glucuronide levels, particularly
when CrCL drops below 25 mL/min. In contrast, within higher
CrCL ranges, a reverse relationship was observed with increased
MPA clearance as renal function improves. In our study, patients
gone from a uremic presentation during the pre-transplant evalu-
May be par-
activity both in PBMCs and in CD4+
cells, which may be par-
expression of both genes fit with the variation in steroid dosage
(Fig. 2 and Table 3).

Since steroids are known to interfere with several gene expres-
pathways by transactivation and transrepression mecha-
nisms, the association between IMPDH1/2 levels and steroids
supports the hypothesis that up regulation is mainly related to the
high steroid dosages. Moreover, the lack of association between
IMPDH activity and mRNA levels suggests that the increase in
gene expression did not immediately lead to an increase in protein
synthesis. Furthermore, although mRNA concentration is com-
monly used to approximate the concentrations and activities of the
pre-transplant time.

No correlation was found between ARs and the main MPA PK/PD
parameters, whereas patients who underwent ARs exhibited
significantly higher pre-transplant IMPDH1 mRNA levels (p =
0.0025; Fig. 3). The rather poor sensitivity and specificity observed in the ROC curve analysis for IMPDH1 suggest that
further studies are needed to better assess this value. Moreover, the
lack of significant differences in IMPDH activity and expression
levels between AR and non-AR patients may be due to the influ-
ence of exogenous factors as steroids that were not supplied at
infections (I). The data are reported as median values; boxes indicate the 25th and 75th percentile, error bars the 10th and 90th percentile.

As previously reported, IMPDH expression did not immediately lead to an increase in protein
transcription, translational and degradation regulation in the deter-
mination of protein concentrations contributes at least as much
as transcription itself. Nevertheless, in our de novo patient popu-
lation an increasing trend in IMPDH activity over time was found
(Fig. 1), although to a lesser extent with respect to the trend
observed in long-term patients. No data for IMPDH gene expres-
sion are available for very long-term patients. However, the very
low dosage or lack of steroids in long-term therapy should preclude
any correlation with an increasing trend in activity, whereas a
correlation with MMF is conceivable. This correlation cannot be
excluded in de novo patients, even though the steroid interference
present in our population does not permit confirmation.

During our study, seven patients experienced an AR episode. No correlation was found between ARs and the main MPA PK/PD
patterns, whereas patients who underwent ARs exhibited
significantly higher pre-transplant IMPDH1 mRNA levels (p =
0.0025; Fig. 3). The rather poor sensitivity and specificity observed in the ROC curve analysis for IMPDH1 suggest that
further studies are needed to better assess this value. Moreover, the
lack of significant differences in IMPDH activity and expression
levels between AR and non-AR patients may be due to the influ-
ce of exogenous factors as steroids that were not supplied at
pre-transplant time.

A higher basal expression of IMPDH isoenzymes reasonably
leads to a higher MPA requirement, to obtain similar immunosup-
pressive effects. From this point of view, the validation of a
transcriptomic assay for pre-transplant IMPDH1 and IMPDH2

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may contribute to prediction of individual drug responsiveness to better assess an effective strategy for MMF dosage adjustment to personalize the immunosuppression.

MPA-related AEs are common in MMF-treated transplant patients. All patients experienced at least one AE. With the exception of the correlation with gastrointestinal AE and totMPA, no significant correlation with PK or PD parameters was found. On the contrary, patients who underwent hematological complications exhibited significantly lower IMPDH1 pre-transplant levels (p = 0.032; Fig. 4), indicating that in patients with low basal IMPDH synthesis, a reduced MMF dosage may avoid this type of AE.

In conclusion, this study suggests that IMPDH1 and IMPDH2 mRNA expression may not represent a useful PD biomarker of MMF during the earliest period after transplant, probably due to the influence of high steroid dosages. However, it is uncertain if in long-term follow-up patients, in whom steroids are reduced or removed, this PD approach might actually be useful. On the other hand, the association we found between pre-transplant levels of IMPDH1 and AR episodes and hematological AE suggests that further studies would be suitable to assess whether these parameters could be an additional predictive tool of efficacy and safety during MMF therapy.

We cannot rule out that the increase of pre-dose IMPDH activity over time and the variability of IMPDH levels post-transplant might also be related to changes in immune cell population function following transplantation (e.g., improvement in uremic state, tapering of immunosuppression), and to the presence of allelic variants of IMPDH1/2 genes, as well. Thus, we are also aware that post-transplant IMPDH activity and/or gene expression should not be abandoned as potential relevant biomarkers.

Despite the low overall number of rejection episodes, the results obtained should encourage further studies on a larger cohort of patients which might shed light on these topics.

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